Topic 6 – Diabetes, lipids, metabolism – B

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0260

Imidazoline I1 receptor ligands activate hepatic adiponectin pathways and thus improve insulin sensitivity

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Metabolic syndrome is defined as a cluster of cardiovascular and metabolic disorders. Previous studies in rat models of metabolic syndrome have demonstrated that ligands selective for I1 imidazoline receptor (LNPs) increase insulin sensitivity through central sympathoinhibition and an additional peripheral effect attributable to adiponectin, a major insulin-sensitizer adipokine. The objective of this study was to explore possible direct actions on hepatocytes, one of the target cells of insulin and adiponectin.

Experiments were carried out in HepG2 cells, a cell line of hepatocytes. In order to evaluate the effect of LNPs on insulin sensitivity, the activation (i.e. phosphorylation) of a key actor of insulin pathways, AKT, was evaluated by measuring the ratio pAKT/AKT by Western Blot. Similarly, the effect of LNPs on adiponectin signaling was evaluated by measuring the rate of phosphorylation of the central kinase involved in adiponectin pathways, AMPK, by Western Blot. Insulin (10 µM) induced the phosphorylation of AKT (pAKT/AKT=0.49±0.16) compared to control without insulin (pAKT/AKT=0.11±0.03; p≤0.05) whereas LNPs (1µM) alone did not. Interestingly, pretreatment by LNPs (1 µM) during 60 min could potentiate the insulin-induced activation of AKT: LNP509: pAKT/AKT=1.13±0.18 (p≤0.05 vs insulin alone); LNP599: pAKT/AKT=1.23±0.16 (p=0.0545 vs insulin alone).

Concerning adiponectin signaling pathways, LNPs alone (from 10^{-9} M to 10^{-4} M) increased AMPK phosphorylation in a concentration- and time-dependent manner. The maximal effect was obtained after 10 min exposure of LNPs 10 µM (untreated cells: pAMPK/AMPK=0.18±0.04; LNP 509 pAMPK/AMPK=0.38±0.05 p≤0.05; LNP599 pAMPK/AMPK=0.46±0.17). These data suggest that LNPs on hepatic cells activate adiponectin pathways and potentiate insulin action. These two direct effects on insulin sensitive cells could account for the ameliorated insulin sensitivity observed in vivo.

0418

Sodium-glucose cotransporters (SGLT) in the heart. Contribution of SGLT-type of transport in hyperglycemia-induced signaling pathway in adult cardiomyocytes

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Background: Exposure to hyperglycemic conditions increases reactive oxygen species (ROS) production in adult cardiomyocytes, inducing glucotoxicity. This is due to an NADPH oxidase activation and more particularly the NOX2 isoform. Our group has demonstrated that hyperglycemia-induced toxic effect does not required glucose metabolism but results from glucose transport through a SGLT type of transport. SGLT acts as glucose sensor. Seven SGLT isoforms have been described (SGLT1 to 6 and SMIT1) but their expression in the heart remains to be elucidated. The aim of this work is to study SGLT isoforms expression in the heart and identify the isoform responsible for glucotoxicity.

Methods: SGLT isoforms expression has been performed in heart extracts (from rats, mice and humans) and in isolated cardiomyocytes (from rats and mice) using PCR and westernblotting. The study of the contribution of each isoforms to glucotoxicity is based on the substrates specifity of all these SGLT isoforms.

Results: SGLT1, SGLT3 and SMIT1 are expressed in the heart and in cardiomyocytes from rats and mice as well as in human heart. In human heart, SGLT3 expression is marginal. SGLT4 is only expressed in rat heart. In presence of 5 mM glucose, rat cardiomyocytes exposure to high concentration of galactose (16 mM, transported through SGLT1) does not activate NOX2. By contrast, myo-inositol (16 mM, transported through SMIT1) completly reproduces hyperglycemic effects. Indeed, it favors p47phox translocation inducing NOX2 activation and stimulates ROS production. This ROS production is blocked by a NOX2 specific inhibitor (gp91dstat). Similar observation was perfomed in mice cardiomyocytes.

Conclusion: SGLT1 and SMIT1 are expressed in rats, mice and human cardiomyocytes. Increased transport through SMIT1 activates NOX2.

0347

Leucine, a potent inhibitor of cardiac glucose uptake

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Background: It was demonstrated that branched-chain amino acids like leucine induce insulin resistance in muscle and adipose tissues. The mechanism proposed to explain leucine action involves mTOR/p70S6K signaling. This pathway can be activated by leucine and is implicated in the stimulation of an insulin negative feedback loop. Knowing that insulin-resistance participates in diabetic cardiomyopathy, we were interested in studying leucine effect in cardiomyocytes.

Methods: Primary cultured adult rat cardiomyocytes were pretreated with different concentrations of leucine (from 1 to 10 mM) during different periods of time (up to 20h) before being exposed to insulin (3x10-9 M, 30 min).

Results: In absence of leucine, insulin induced a 6-fold increase in glucose uptake (0.31+/-0.04 vs. 0.05+/-0.01 µmoles/mg.h). This correlated with the increase in phosphorylation state of PKB and AS160, both known to regulate glucose transport downstream of insulin. Pre-incubation with leucine for 1 h stimulated mTOR/p70S6K pathway resulting in the inhibiting phosphorylation of IRS-1 located in the proximal insulin signaling pathway. This is accompanied by a significant decrease in PKB and AS160 phosphorylation but, surprisingly, insulin-stimulated glucose uptake was preserved (0.31+/–0.05 µmoles/mg.h). On the other hand, a longer incubation (14h) with leucine induced a drastic decrease in glucose transport (0.056+/–0.01 µmoles/mg.h). The mTOR/p70S6K inhibitor rapamycin did not prevent this inhibition. Moreover, the non-metabolized leucine analog BCH was able to stimulate mTOR/p70S6K pathway but had no effect on the insulin-mediated stimulation of glucose uptake. By contrast, intermediates of leucine catabolism, alpha-ketoisocaproate, acetoacetate and beta-hydroxybutyrate, inhibited glucose uptake similarly to leucine.

Conclusion: Leucine catabolism reduces insulin-dependent glucose transport independently of insulin signaling.

0447

Elevated plasma PCSK9 is equally detrimental for non-familial hypercholesterolemic (non-FH) and heterozygous FH patients, irrespective of their LDL receptor defects

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Objectives: Do elevated PCSK9 levels constitute an even greater risk for people who already have reduced LDL receptor (LDLR) levels, such as heterozygous familial hypercholesterolemic (HeFH) patients?

Methods: Circulating PCSK9 was measured by ELISA in non-treated HeFH patients carrying either a D206E (n=237), V408M (n=117), or D154N (n=38) LDLR missense mutation and in normolipidemic controls (n=152). Skin fibroblasts and lymphocytes were isolated from a subset of patients and grown in 0·5% serum and mevastatin with increasing amounts of recombinant PCSK9. LDLR abundance at the cell surface was determined by flow cytometry.